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Response of single nephron glomerular filtration rate to distal nephron microperfusion

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Response of single nephron glomerular filtration rate to distal nephron microperfusion. The observation that single nephron glomerular filtration rate (SNGFR) measured with proximal tubular collections is higher than SNGFR based on distal tubular collections has been attributed to the blockade of distal delivery during proximal collections, thus leading to an altered status in a distal nephron feedback mechanism thought to participate in the control of SNGFR. This hypothesis was tested further by evaluating SNGFR (measured from complete early proximal tubule collections) in the presence or absence of distal nephron perfusion via a late proximal nephron site. SNGFR measurements were also obtained from other nonperfused tubules selected at random. Artificial tubular fluid (ATF), Ringer's solution with or without calcium, or 0.9% sodium chloride solution was perfused at 25 nl/min; at least two solutions were used in nine of the 19 dogs. When distal delivery was maintained with ATF (19 tubules, seven dogs), SNGFR averaged 45 ± 6 nl/min and rose to 72 ± 9 nl/min following cessation of perfusion. SNGFR in other nonperfused tubules was 72 ± 6 nl/min. During distal perfusion with Ringer's solution (21 tubules, eight dogs), SNGFR was 52 ± 7 nl/min and was also significantly lower than SNGFR in nonperfused tubules (76 ± 9 nl/min). Thus, SNGFR was lower when distal perfusion was maintained with ATF or Ringer's solution. In contrast, when 0.9% NaCl was used as the perfusion solution (19 tubules, seven dogs), SNGFR averaged 71 ± 9 nl/min and did not differ significantly from SNGFR in nonperfused tubules (75 ± 6 nl/min). During perfusion with calcium-free Ringer's solution (15 tubules, six dogs), SNGFR was 76 ± 12 nl/min and it, also, did not differ significantly from SNGFR in nonperfused tubules (82 ± 11 nl/min). The results support the concept that one or more functions of distal delivery participate in the control of SNGFR and they suggest that at least one of these functions may be reflected by changes in distal calcium delivery.

Réponse du débit de filtration glomérulaire du néphron à la microperfusion de son segment distal. Le fait que le débit de filtration glomérulaire individuel d'un néphron (SNGFR) mesuré par une collection tubularie proximale est plus élévé que le SNGFR mesuré par une collection tubulaire distale a été attribué à l'arrêt de l'écoulement dans le distal pendant une collection proximale, ce qui entraînerait une altération des mécanismes de feedback du néphron distal que l'on pense participer au contrôle de SNGFR. Cette hypothèse a été examinée plus avant par la mesure de SNGFR (à partir d'une collection proximale complète) en présence ou en l'absence d'une perfusion du néphron distal assurée à partir d'un site proximal tardif. Des mesures de SNGFR ont aussi été obtenues à partir d'autres tubules, non perfusés et sélectionnés au hasard. Du liquide tubulaire artificiel (ATF), du Ringer avec ou sans calcium ou du NaCl à 0,9% ont été perfusés à 25 nl/min; au moins deux solutions ont été utilisées chez 9 des 19 chiens. Quand le débit distal est maintenu avec de l'AFT (19 tubules, 7 chiens), le SNGFR est en moyenne de 45 ± 6 nl/min et augmente à 72 ± 9 nl/min quand on cesse la perfusion. SNGFR dans les autres tubules non perfusés est de 72 ± 6 nl/min. Au cours de la perfusion distale avec du Ringer (21 tubules, 8 chiens), le SNGFR est de 52 ± 7 nl/min, significativement inférieur à la valeur obtenue dans les tubules non perfusés (76 ± 9 nl/min). Ainsi le SNGFR est-il inférieur quand une perfusion distale est maintenue avec de l'AFT ou du Ringer. Au contraire; quand de NaCl 0,9% est perfusé (19 tubules, 7 chiens) SNGFR est en moyenne de 71 ± 9 nl/min et n'est pas significativement différent de des valeurs obtenues dans les tubules non perfusés (75 ± 6 nl/min). Au cours de la perfusion par un Ringer sans calcium (15 tubules, six chiens), SNGFR est de 76 ± 12 nl/min, et, là encore, n'est pas significativement différent de SNGFR des tubules non perfusés (82 ± 11 nl/min). Ces résultats sont en faveur de l'idée qu'une ou des fonctions de la charge délivrée au distal participeraient au contrôle de SNGFR et ils suggèrent qu'au moins l'une de ces fonctions pourrait être traduite par des modifications de la charge de calcium délivrée au distal.

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The results of renal micropuncture experiments in dogs have demonstrated that measurements of single nephron glomerular filtration rate (SNGFR) that are derived from complete collections of proximal tubule fluid are higher than those that are based on complete collections of distal tubule fluid [1]. This quantitative difference between measurements of SNGFR from proximal vs. distal collection sites has been ascribed to technical interference with the delivery of tubule fluid to the distal nephron during proximal collections. The interposition of an intralumenal oil block between the site of proximal collection and the remainder of the nephron effects a reduction in distal delivery, some function of which may serve to activate a distal nephron feedback signal (presumably generated within the region of the macula densa) that participates in the control of SNGFR. In this setting, afferent arteriolar

tone might thereby be diminished and SNGFR would be expected to rise.

While smaller in magnitude, a quantitative difference in proximal vs. distal measurements of SNGFR has also been described in the rat [2, 3]. On the other hand, such a difference has not been observed by all investigators and the existence of a distal nephron feedback mechanism for the regulation of SNGFR has been questioned in this species [4]. The results of studies of the influence of *in vivo* microperfusion of the loop of Henle and the distal nephron on SNGFR in the rat have been similarly conflicting. In some experiments, an increased rate of microperfusion of the distal nephron with modified Ringer's solution (via a puncture site in the late proximal convoluted tubule) has been accompanied by a simultaneous reduction of SNGFR (as measured from an earlier proximal collection site) or proximal lumenal stop-flow pressure [5–7]. In other experiments, increased distal microperfusion (as reflected by the rate of volume flow at earlier proximal collection sites) has not been attended by any alteration of SNGFR [8]. Furthermore, in the rat, SNGFR and proximal lumenal stop-flow pressure have been reported to increase minimally and inconsistently when distal delivery is reduced to values below that observed under usual physiological conditions [6, 7, 9, 10].

Thus far, a completely satisfactory explanation for these discrepant observations in the rat (and between rat and dog) has not been provided. Recent observations in the dog have emphasized that differences between proximal and distal measurements of SNGFR are most apparent when renal perfusion pressure is relatively high (approximately 140 mm Hg) and overall renal autoregulatory capability has been clearly shown to be intact [1]. Although not yet established with certainty, the possibility of differences among species or strains, technical methodologies and physiological conditions of study (level of renal perfusion pressure, presence or absence of an intact autoregulatory capability, differing capacities to effect an adjustment of afferent arteriolar resistance as a function of differences in salt balance, extracellular fluid volume, etc.) must be acknowledged.

Irrespective of the eventual explanation for the discrepant observations in the rat, our earlier description of a relatively large difference in proximal vs. distal measurements of SNGFR in the dog (and the availability of abundant information on the nature of renal autoregulation in this species) suggested that further evaluation of the nature and contribution of a distal nephron feedback mechanism to the control of SNGFR in this species would be of interest. Accordingly, the present renal micropuncture studies were initiated in the dog to ascertain the single nephron response to the intralumenal microperfusion of individual distal nephrons (via a late puncture site in the proximal convoluted tubule). Primarily, we wished to determine whether or not the maintenance of distal perfusion during simultaneous proximal collections (at earlier puncture sites) would prevent or minimize the usual elevation of SNGFR. If so, we also desired to evaluate to what extent such an effect might be mediated as a function of the solute composition of the distal perfusate or its rate of volume delivery to the distal nephron.

Methods

Simultaneous renal micropuncture and clearance studies were performed in 19 mongrel dogs of either sex (body wt, 18 to 22 kg). Anesthesia was induced by the i.v. administration of sodium pentobarbital (30 mg/kg) and small supplemental doses were administered as necessary throughout the experiment. A cuffed endotracheal tube was introduced and the animals were allowed to ventilate spontaneously on room air. Thereafter, the dogs were prepared for simultaneous renal clearance and micropuncture studies as described in detail previously [1]. In brief, a peripheral foreleg vein was first catheterized for the administration of a priming dose of inulin followed by a sustaining infusion at a rate sufficient to maintain a plasma concentration of approximately 100 mg/100 ml. A catheter was inserted through one femoral artery into the aorta to a level slightly below the left renal artery and then connected to a pressure transducer (Statham, Statham Laboratories, Inc., Hato Rey, Puerto Rico). This catheter was utilized for sequential monitoring of the sytemic arterial blood pressure and the collection of arterial blood samples. Another catheter was inserted through the contralateral femoral artery so that its tip was placed just above the level of the renal arteries. This catheter was used for the injection of 1- to 2-ml volumes of a 10% solution of lissamine green dye to facilitate the identification of early and late segments of proximal convoluted tubules. The left kidney and renal artery were then exposed and isolated via a flank incision. An electromagnetic flow transducer (Carolina Medical Electronics, Inc., King, NC) was then placed around the renal artery (whenever a single artery was present) in order that renal blood flow could be monitored directly and continuously via a recorder (Sanborn). The left ureter was then catheterized for timed and sequential collections of urine from the experimental kidney. The left kidney was mounted thereafter on a plastic holder (Lucite) and its surface was prepared for micropuncture. Prior to micropuncture, the autoregulatory ability of each kidney was assessed

by briefly clamping the renal artery and noting the temporary but typical hyperemic phase of the vascular recovery pattern following the release of constriction [11].

Micropuncture and microperfusion studies were initiated approximately 45 min following administration of the priming dose of inulin. In preparation for measurements of SNGFR from proximal collection sites in the presence or absence of simultaneous microperfusion of the distal nephron (via a puncture site in a later segment of the same proximal tubule), an early segment of a proximal convoluted tubule was first identified by one of two methods: *1*) observance of the passage of lissamine green dye along the length of a proximal tubule following its intraaortic injection or, more frequently, *2*) random puncture of a proximal convolution with a small-tipped pipet (1 to 2 μ O.D.) that was filled with nigrosin dye, and subsequent observation of the direction of tubule fluid flow according to the intralumenal passage of a small volume of injected dye along later segments of the same tubule. When later segments could be identified by the latter technique, the nigrosin-filled pipet was left in place and a perfusion pipet was introduced into a selected downstream segment whose long axis was parallel to that of the perfusion pipet. The perfusion pipets were filled with test solutions of differing composition (see following) and attached by a 0.5-cm length of polyethylene tubing (PE 100) to a stainless steel tube which was connected to a 10 μl glass microsyringe, both of which were filled with mineral oil. The rate of perfusion was controlled with a syringe infusion pump (Sage Instruments, Model 355). The infusion rate was adjusted to 25 nl/min, a figure that approximates the usual flow of late proximal fluid in a species whose actual SNGFR probably approximates 50 nl/min [1]. All perfusion solutions were tinged lightly with lissamine green dye (0.5%) and the adequacy of microperfusion was first assessed for a brief period following the initiation of perfusion. Tubules were not studied further if visible leakage of the perfusion solution was observed at the site of puncture, if the passage of solution along the length of more distal convolutions was not observed, if it appeared to be discontinuous for whatever reason or if retrograde perfusion of the tubule was noted. When none of these phenomena were observed during the initial period of microperfusion, and the downsteam flow of perfusate appeared to be even and uniform, the more proximally placed and nigrosin-filled pipet was withdrawn and its site of insertion was marked by the intraepithelial deposition of nigrosin dye. Immediately thereafter, an oil-filled (Sudan Black-stained mixture of olive and silicon oils) collection pipet (tip diameter, 3 to 6 μ O.D.) was inserted (against the direction of tubule fluid flow) into the earlier proximal segment via the same nigrosin-marked puncture site. The relative positions of the collection and perfusion pipets are shown in Fig. 1. A column of oil sufficient to fill the tubule lumen to a distance of approximately 4 to 5 tubule diameters was then injected, and a timed collection of proximal fluid (one to two minutes in duration) was initiated immediately thereafter for measurement of SNGFR during the continued maintenance of distal nephron perfusion. The delay in time between the injection of the oil column and the initiation of sampling was kept to a minimum. Whenever necessary, a slight negative pressure was applied to initiate the collection which then proceeded without continuous aspiration. Intermittent negative pressure was used when necessary to maintain the oil column in constant position throughout the collection period. Particular care was taken to insure that the oil droplet did not move downstream beyond the more distal position of the perfusion pipet; a new tubule was selected for study whenever this occurred. Following the completion of timed collections of proximal fluid, distal perfusion was discontinued and both the collection and perfusion pipets were removed from the

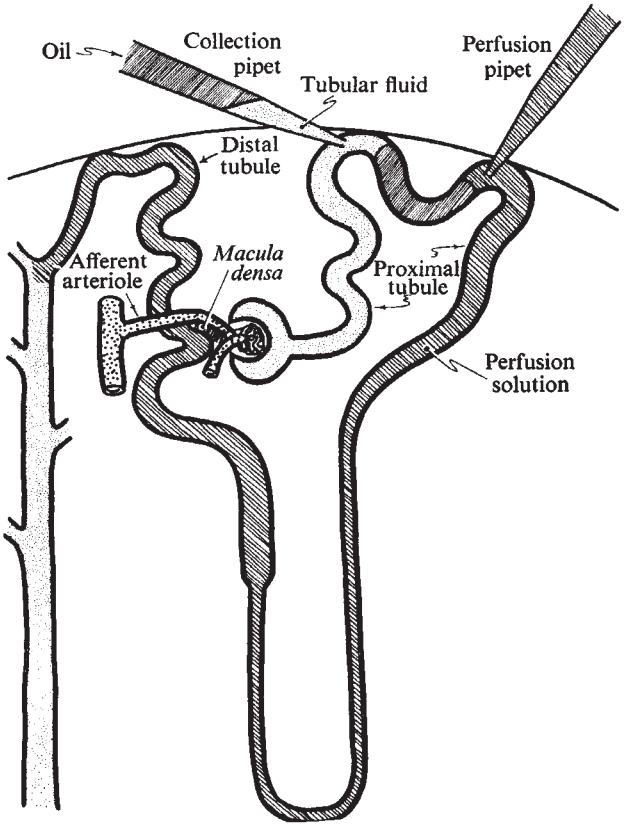

Fig. 1. *Schematic representation of nephron with a perfusion pipet in late proximal convolution and a collection pipet in an earlier proximal segment.*

tubule. Upon removal of the collection pipet, a small volume of mineral oil on the surface of the kidney was aspirated into its tip. A second oil-filled collection pipet with a larger tip diameter (5 to 10 μ O.D.) was reintroduced into the same nigrosin-marked early puncture site. An oil column was again introduced into the tubule lumen and a second timed collection of proximal fluid was obtained from the same tubule in the absence of distal perfusion. Second collections also ranged between one and two minutes in duration and, for the most part, the technical criteria outlined by Wright and Giebisch were again fulfilled [12].

Upon completion of the second proximal collection (in the absence of distal perfusion) from the same tubule, a surface convolution of a different proximal tubule was selected at random for a single measurement of SNGFR in a nonmanipulated and nonperfused tubule. In each kidney, the preceding experimental sequence was repeated two or three times with a single perfusion solution, i.e., *1*) measurement of proximal SNGFR during the maintenance of distal perfusion; *2*) measurement of proximal SNGFR in the same tubule following the cessation of distal perfusion; *3*) measurement of proximal SNGFR in a different tubule only in the absence of distal perfusion. However, in nine of the 19 animals, the same experimental sequence was repeated an additional two or three times utilizing a second perfusion solution of differing composition. The micropuncturist was not informed as to the nature of the perfusion solution during any of the experiments.

Upon completion of the study, the perfusion rate for each pipet was validated *in vitro* by immersion of its tip into a siliconized Petri dish filled with hydrated mineral oil and deposition of at least two or three timed deliveries on the bottom of the dish (utilizing the same pump setting as that used during *in vivo* perfusion). The volume of each bubble of perfusion solution was then aspirated and measured in a constant-bore capillary pipet using a micrometer slide comparator (Gaertner Scientific Corporation, Chicago, IL).

Three or four timed collections of urine were obtained sequentially throughout the period of micropuncture manipulation. Samples of arterial blood were obtained at the approximate mid-point of each urine collection period (usually occurring with every second collection of proximal fluid). Inulin clearance measurements were utilized as measures of glomerular filtration rate. In addition, renal blood flow and systemic arterial pressure were monitored directly throughout each study.

Four perfusion solutions of differing composition were utilized during this study (Table 1): *1*) the first solution, arbitrarily termed "artificial tubule fluid" (ATF), which contained sodium, potassium, magnesium, chloride, bicarbonate, phosphate, sulfate, urea and dextrose in the concentrations as outlined in Table 1; *2*) isotonic sodium chloride solution (145 mEq/liter); *3*) modified Ringer's solution similar to that utilized in earlier studies by Schnermann et al [5]; and *4*) a modified Ringer's solution whose composition differed from solution No. 3 only in that it did not contain $CaCl_2$. This solution was termed "calcium-free Ringer's solution". All solutions were tinged lightly with lissamine green (0.5%), and each solution was filtered (Millipore filter) immediately prior to use.

The tubule fluid volume was measured with a calibrated micropipet using a slide comparator and the tubule fluid inulin concentration was measured in triplicate using a microfluorometric method [13] whose boiling time was modified to ten minutes. Inulin concentrations in plasma and urine were measured using an anthrone colorimetric technique. Microhematocrit measurements were performed on all arterial blood

Table 1. Composition of solutions for microperfusion[a]

Solute	AFT	Saline solution	Ringer's solution	Calcium-free Ringer's solution
Na^+, *mEq/liter*	140	145	140	140
K^+, *mEq/liter*	4	—	4	4
Ca^{++}, *mEq/liter*	4	—	4	—
Mg^{++}, *mEq/liter*	2	—	—	—
Cl^-, *mEq/liter*	120	145	138	134
HCO_3^-, *mEq/liter*	25	—	10	10
$HPO_4^=$, *mEq/liter*	2	—	—	—
$SO_4^=$, *mEq/liter*	2	—	—	—
Urea, *mg/100 ml*	25	—	45	45
Dextrose, *mg/100 ml*	50	—	—	—

[a] All solutions were tinted lightly with 0.5% lissamine green. ATF, artificial tubule fluid.

samples. Plasma protein concentrations were determined by light refractometry (American Optical Corporation, Scientific Instruments Division, Buffalo, NY). SNGFR was calculated as the product of the volume flow of tubule fluid (nl/min) and the ratio of the tubule fluid to plasma inulin concentration (TF/P). Sodium concentrations in plasma and urine were measured by flame photometry.

At the termination of each experiment, the electromagnetic blood flow transducer was calibrated directly *in situ* by catheterizing the renal artery and obtaining a timed collection of blood. The left kidney was then excised, stripped of fat and surrounding tissue, blotted dry and weighed. Standard statistical tests were applied to the data and both the unpaired and paired *t* tests were used to assess the statistical significance of differences [14].

Results

Hemodynamic and clearance data. Average values for systemic blood pressure, renal blood flow, inulin clearance, urine flow, urine sodium excretion, plasma protein concentration, dog and kidney weight and hematocrit are shown in Table 2. These values were comparable at the time that distal microperfusion was performed with each of the four test solutions.

Distal perfusion with ATF (Fig. 2). SNGFR was measured during the simultaneous perfusion of ATF into 19 late proximal tubules of seven dogs. Re-collection measurements of SNGFR were obtained in 15 of these same tubules following the cessation of perfusion and, in addition, 14 measurements of SNGFR were obtained from other nonperfused proximal tubules that were selected randomly. The average SNGFR (derived from the sum of all measurements in individual tubules) during simultaneous perfusion with ATF was

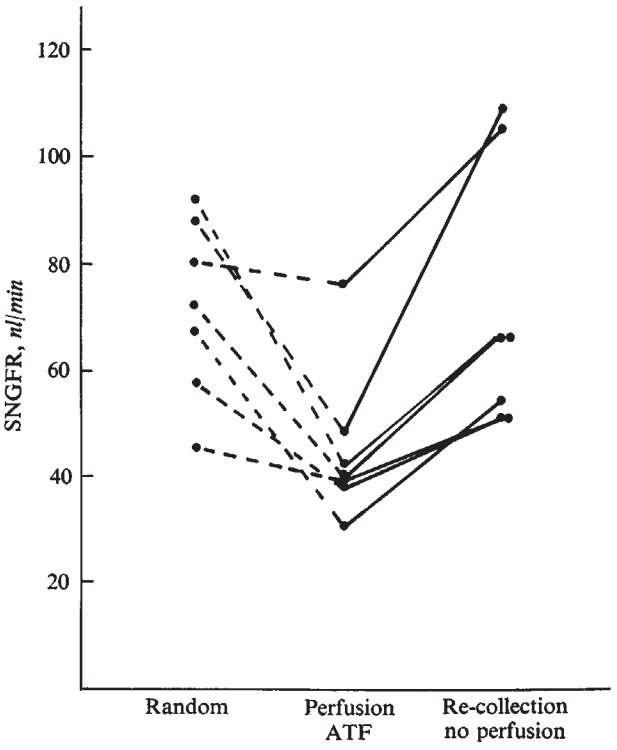

Fig. 2. *Effect on SNGFR of distal microperfusion with artificial tubular fluid.* Each *point* represents the average values from individual dogs. The *solid lines* connect values from the same nephron during microperfusion and following its cessation. The *dotted lines* connect values during microperfusion with values from other nonperfused tubules that were selected at random after each re-collection without perfusion had been completed.

42 ± 4 (mean $\pm$ SEM) nl/min, a value significantly lower ($P < 0.01$) than that observed in the same tubules following the cessation of perfusion (69 ± 7 nl/min), or that measured in other nonperfused tubules that were randomly selected (72 ± 5 nl/min). Average values for

Table 2. Hemodynamic and clearance data during distal microperfusion[a]

Perfusate	BP	RBF[b]	C_{In}[b]	V[b]	$U_{Na}V$[b]	$P_{protein}$	Weight		%
							dog	kidney	
	mm Hg	*ml/min*	*ml/min*	*ml/min*	*μEq/min*	*g/100 ml*	*kg*	*g*	
ATF	120	161	28	0.22	29	6.1	21	51	40.4
($N=7$)	±6	±24	±2	±0.06	±10	±0.2	±1	±3	±1.9
Isotonic saline	123	202	35	0.42	50	6.0	20	44	40.0
($N=7$)	±6	±22	±3	±0.07	±11	±0.2	±1	±3	±1.4
Ringer's	122	194	31	0.40	30	6.2	20	45	44.6
($N=8$)	±8	±20	±3	±0.06	±8	±0.3	±2	±5	±1.6
Ca^{++}-free Ringer's	134	195	33	0.39	36	6.0	21	47	43.3
($N=6$)	±6	±17	±3	±0.06	±8	±0.3	±2	±5	±1.4

[a] Values are mean ± SEM. BP, blood pressure; RBF, renal blood flow; C_{In}, inulin clearance; V, urine flow; $U_{Na}V$, urine sodium excretion; Hct, hematocrit; *N*, number of dogs.
[b] Data from micropunctured kidney alone.

individual tubules did not differ significantly from those derived from the use of mean values from each dog. The latter values are depicted in Fig. 2. During distal perfusion with AFT, the mean SNGFR (computed from the average values for each kidney) was 45 ± 6 nl/min, a value that was significantly lower ($P < 0.01$) than that observed in the same tubules in the absence of perfusion (72 ± 9 nl/min), or that noted in other nonperfused tubules that were selected at random (72 ± 6 nl/min). Thus, following the cessation of perfusion, SNGFR increased by 62%, and this increase could be related almost entirely to a substantial increase in the rate of volume flow at the site of proximal sampling (37 ± 6 vs. 63 ± 10 nl/min). Absolute reabsorption was not altered significantly (9.5 vs. 10.8 nl/min), TF/P inulin ratios decreased significantly from 1.26 to 1.17 ($P < 0.05$) and fractional reabsorption decreased from 21 to 15%.

Distal perfusion with isotonic saline solution (Fig. 3). During distal perfusion of late proximal tubules with isotonic saline solution, 19 measurements of SNGFR were obtained in seven dogs; an equal number of measurements was obtained from the same tubules following cessation of perfusion. In addition, 15 measurements of SNGFR were obtained from other nonperfused tubules that were selected at random. SNGFR in these 19 tubules averaged 69 ± 6 nl/min during simultaneous perfusion with isotonic saline solution. This average value was not significantly different from that observed in other nonperfused tubules that were randomly selected (79 ± 5 nl/min). However, upon the termination of distal perfusion in the same nephron, SNGFR rose slightly to an average value of 83 ± 5 nl/min. These mean data from individual tubules were similar to those obtained when average values from each dog were used (Fig. 3). In the latter circumstance, SNGFR averaged 71 ± 9 nl/min during distal perfusion, a value similar to that of the other randomly selected and nonperfused proximal tubules (75 ± 6 nl/min) but significantly lower (paired analysis) than that noted in the same tubules after cessation of distal perfusion (84 ± 8 nl/min). The slight increase in SNGFR upon the cessation of perfusion was accompanied by a nearly parallel increase in the volume flow of proximal fluid (58 ± 8 vs. 75 ± 9 nl/min). However, this observed increase in SNGFR was relatively modest (+23%) as compared to that observed during perfusion with ATF.

Distal perfusion with Ringer's solution (Fig. 4). Perfusion of late proximal tubules with a calcium-containing Ringer's solution was performed in 21 tubules in

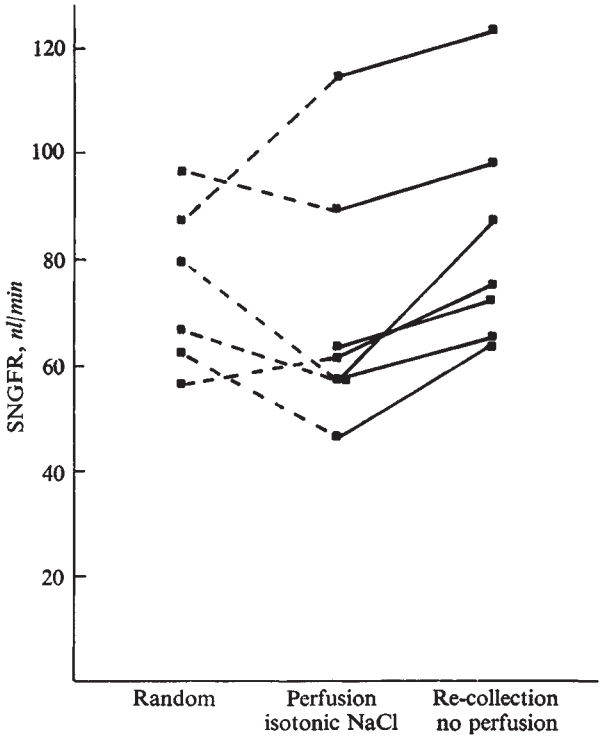

Fig. 3. *Effect on SNGFR of distal microperfusion with isotonic saline solution.* Individual *points* reflect average values from seven dogs. SNGFR during perfusion does not differ significantly from values observed in nonperfused tubules.

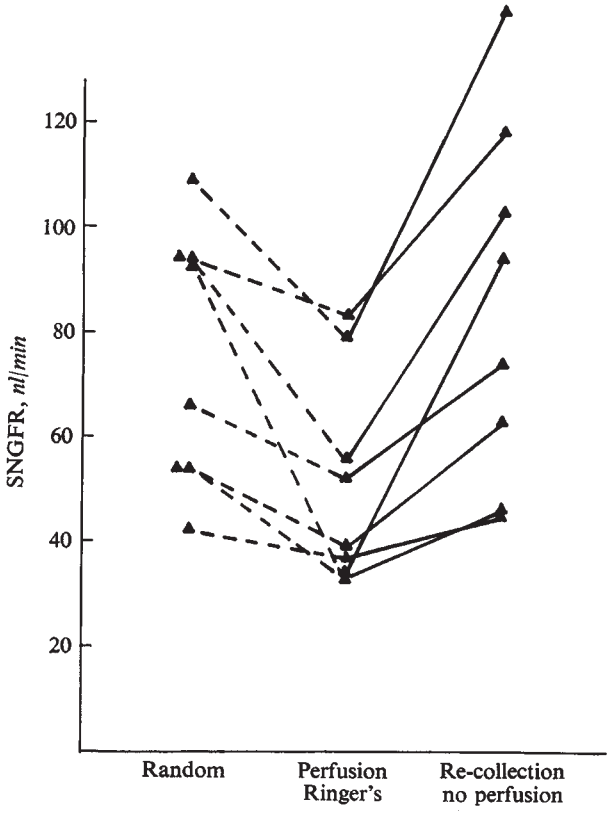

Fig. 4. *Effect on SNGFR of microperfusion with calcium-containing Ringer's solution.* Individual *points* represent the average values from each of eight dogs. SNGFR during perfusion was significantly lower than that observed in the absence of perfusion, either in the same or different nephrons.

eight dogs; re-collection measurements were obtained from 20 of these same tubules following the cessation of perfusion. Single measurements of SNGFR were obtained from 21 other nonperfused proximal tubules that were selected randomly. During distal perfusion, SNGFR in these individual 21 tubules averaged 53 ± 5 nl/min. Following the cessation of perfusion, re-collection measurements of SNGFR from the same tubules averaged 85 ± 9 nl/min. The average re-collection SNGFR was similar to that observed in other nonperfused and randomly selected tubules (77 ± 6 nl/min). The results were almost identical when the mean data from each dog were used (Fig. 4). In the latter circumstance, the mean SNGFR during distal perfusion with calcium-containing Ringer's solution averaged 52 ± 7 nl/min, a value significantly lower ($P < 0.01$) than that noted in the same tubules following the cessation of perfusion (86 ± 12 nl/min) and that observed in other tubules selected at random (76 ± 8 nl/min). Thus, upon the cessation of perfusion with calcium-containing Ringer's solution, SNGFR increased by 68% and this effect was accompanied by a parallel increase in volume flow (40 to 61 nl/min). The average TF/P inulin ratio decreased significantly ($P < 0.05$) from 1.33 to 1.16. Thus, absolute reabsorption did not change significantly (12.9 vs. 11.9 nl/min).

Distal perfusion with calcium-free Ringer's solution (*Fig. 5*). In six dogs, 15 late proximal tubules were perfused with a calcium-free Ringer's solution. In contrast to observations during distal perfusion with calcium-containing Ringer's perfusate, SNGFR during perfusion with this solution was not significantly lower than that measured on random sampling from other nonperfused proximal tubules. Using data from individual tubules, SNGFR during perfusion averaged 79 ± 8 nl/min as compared to an average value of 86 ± 8 nl/min during 13 measurements from other randomly selected and nonperfused proximal tubules. Following the cessation of perfusion, measurements of SNGFR from 15 of the same tubules previously perfused with calcium-free Ringer's were slightly but significantly higher (98 ± 11 nl/min). Average values based on the mean figures from each of six dogs were similar, i.e., SNGFR averaged 76 ± 12 nl/min during perfusion as compared to a value of 82 ± 11 nl/min in other randomly selected and nonperfused proximal tubules whereas, following the cessation of perfusion, SNGFR in the same tubules averaged 96 ± 15 nl/min. The increase in SNGFR during re-collection measurements from the same tubules, although significant statistically ($P < 0.05$), averaged only +26%, a fractional increase that was substantially less than comparable changes that were observed upon cessation of perfusion with ATF or calcium-containing Ringer's solution.

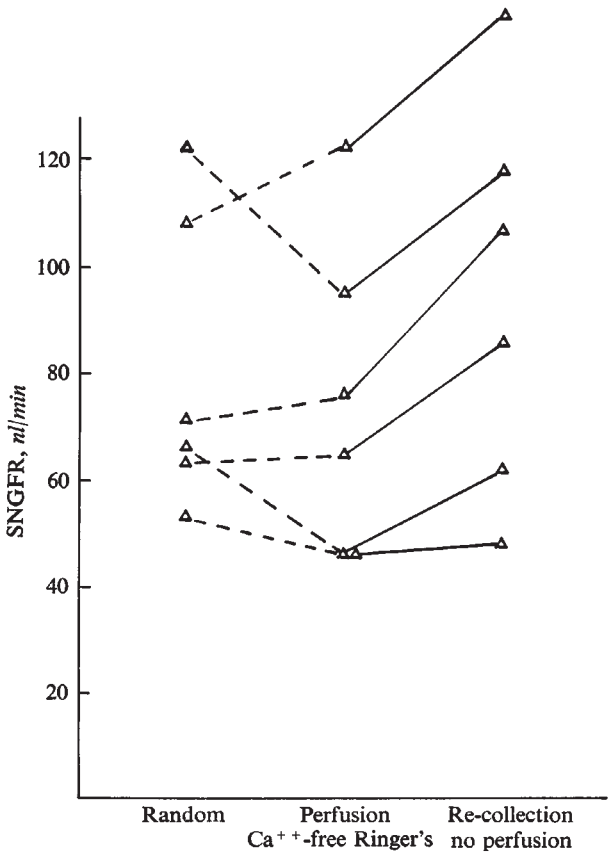

Fig. 5. *Effect on SNGFR of distal microperfusion with calcium-free Ringer's solution.* SNGFR during perfusion was not significantly different from that observed in different nonperfused tubules that were randomly selected, but it was slightly lower than values observed in the same nephron in the absence of perfusion.

Summary values for observed changes of SNGFR during distal perfusion with each of the four test solutions are depicted in Fig. 6.

Fig. 7 shows the relative differences in SNGFR during the presence and absence of distal perfusion with the four different solutions. For this plot, the fractional differences observed in each dog were calculated and these differences were averaged. In essence, when tubules were perfused with either ATF or calcium-containing Ringer's solution, measurements of SNGFR in the same tubules following cessation of perfusion or in other nonperfused tubules were 55 to 68% higher than values for SNGFR obtained during the simultaneous maintenance of distal perfusion. On the other hand, during perfusion with isotonic saline or calcium-free Ringer's solution, SNGFR was not significantly different from measurements of SNGFR in other nonperfused tubules selected at random. Furthermore, while re-collection measurements of SNGFR from the same tubule after termination of perfusion were signi-

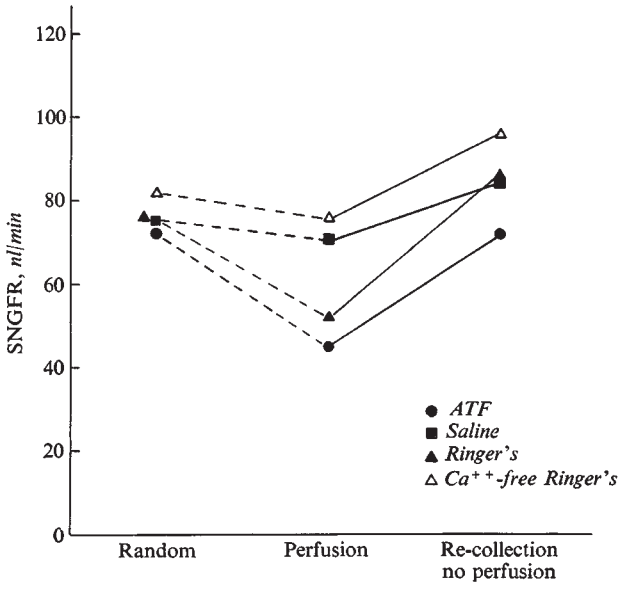

Fig. 6. *Average SNGFR values obtained with each of four test solutions.*

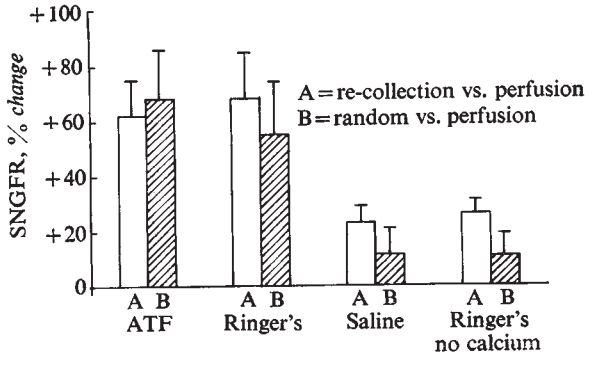

Fig. 7. *Average expression of the effect on SNGFR of distal microperfusion with four different test solutions.* For each perfusion solution, column A compares the average SNGFR during perfusion with the re-collection from the same tubule following the cessation of perfusion, column B compares SNGFR obtained during perfusion with that measured in different nonperfused tubules selected at random. Values are mean ± SEM.

ficantly higher than comparable measurements during simultaneous distal perfusion, the relative increase was no more than 23 to 26%, values that were substantially less than those observed in experiments in which ATF or calcium-containing Ringer's solution were utilized.

Discussion

The present studies were initiated to assess whether or not the maintenance of distal nephron perfusion would minimize or prevent the usual elevation of SNGFR that has been associated with the cessation of distal delivery during complete collections of tubule fluid from proximal puncture sites in the dog [1]. The results demonstrate that distal lumenal perfusion (via a late proximal puncture site at a rate approximating that which occurs normally [25 nl/min]) with either ATF or calcium-containing Ringer's solution is accompanied by simultaneous measurements of SNGFR that are substantially lower than those observed in the same tubule following the cessation of distal perfusion, or in other nonperfused nephrons chosen at random from the surface of the kidney. These measurements of SNGFR (from proximal collections sites) during the maintenance of distal perfusion were quantitatively similar to those that have been derived previously from complete collections of distal tubule fluid when distal delivery was intact [1]. Consequently, in themselves, the results of these studies provide additional support for the suggestion that some function of the rate of fluid delivery to the distal nephron may well participate in the regulatory feedback control of glomerular filtration, i.e., reduced delivery may be attended by an elevation of SNGFR, and vice versa. If so, it is reasonable to suspect that such a feedback mechanism may be mediated via an interplay of signals between the macula densa of the distal tubule and the adjacent afferent arteriole.

The present observations are in general agreement with those obtained earlier during certain perfusion studies in the rat [5], although certain differences can be noted as well. In the present studies, a decrease in distal delivery (from an approximate normal value of 25 nl/min to one approaching zero) was associated with a significant increase in SNGFR. Based on observations in the rat, it has been suggested that the relationship between changes of SNGFR and distal delivery is nonlinear, i.e., decreased delivery below that observed normally effects no more than a slight or negligible rise of SNGFR, while increased delivery to values above normal leads to a disproportionately greater reduction of SNGFR and elevation of proximal intralumenal stop-flow pressure [6, 7, 10]. It is possible that the nonlinearity of this relationship in the rat might be related to the fact that a decrease in distal delivery would not effect a reduction in afferent arteriolar resistance (reflected as an increase in SNGFR) unless the experimental preparation still possessed residual autoregulatory capability. Thus, if afferent arteriolar tone was at or near its minimal level (perhaps due to compromised autoregulatory capability or levels of arterial blood pressure that approach the lower limits of the autoregulatory range), one would not expect that a decrease in distal delivery would lead to a further decrease in afferent arteriolar tone; consequently, alterations in SNGFR might not be

observed in response to the interposition of a proximal intralumenal oil block. In contrast, the present observations in the dog demonstrated clearly that a reduction of distal lumenal perfusion from approximately 25 nl/min to a value approaching zero was accompanied by an obvious increase of SNGFR. The arterial blood pressure in these animals was well within the autoregulatory range and their autoregulatory capability seemed to be intact; hence, the results of the present studies also imply that important quantitative differences may well exist between the autoregulatory behavior of dog and rat. To our knowledge, whole kidney relationships between arterial blood pressure and renal blood flow and/or GFR have not yet been delineated completely in the healthy rat.

The nature of the specific stimulus which mediates the distal nephron feedback response has been the subject of much investigation. Initially, it was suggested that an increased osmolality of distal tubule fluid might serve to effect a reduction of afferent arteriolar resistance [11, 15]. Later, a sodium-linked feedback hypothesis was proposed which differed from that described previously only in the sense that some function of an increase in distal tubule fluid sodium concentration or delivery rate might lead to afferent arteriolar constriction [16]. Specifically, it was suggested that changes in sodium influx into the cells of the macula densa (or its concentration in distal reabsorbate) might lead to an alteration of renin secretion by the single nephron, and that afferent arteriolar tone might then be modulated via a change in the local generation of angiotensin II [17, 18]. Conversely, it was also suggested that afferent vasoconstriction might be mediated via decreased rather than increased sodium transport by the macula densa cells [19, 20]. However, firm evidence in support of one or another specific hypothesis has not yet been presented. In view of the lack of definitive evidence as to the specific nature of any distal feedback mechanism that might exist, it seemed logical to us that additional insight might be obtained by an evaluation of the response of proximal measurements of SNGFR to distal nephron perfusion with solutions of differing solute composition, but similar rates of delivery.

In the present studies, the possible role of changes in the distal delivery of sodium chloride *per se* was evaluated during distal lumenal perfusion with isotonic sodium chloride solution alone. In view of the results of previous experiments suggesting that some function of distal sodium delivery might be of primary importance to operation of the feedback system [5, 7, 16, 21], it was surprising to note that measurements of SNGFR during the simultaneous maintenance of distal perfusion with isotonic sodium chloride were similar to those observed in other nonperfused tubules chosen at random. Distal lumenal perfusion with sodium chloride solution did not prevent activation of the feedback mechanism and the usual elevation of proximal SNGFR that was observed in response to the interposition of a proximal oil block and the consequent decrease of distal delivery. In one sense, these observations demonstrated that the converse response (i.e., the apparent absence of a feedback-mediated elevation of SNGFR) during the maintenance of distal perfusion with ATF or calcium-containing Ringer's solution was not related to a nonspecific effect of perfusion *per se*. At least, the divergence of the SNGFR response to distal perfusion with solutions of differing composition suggested that some function of sodium chloride delivery did not serve as the sole mediator of the feedback response. Furthermore, in view of the fact that comparable rates of perfusion were utilized, the feedback stimulus did not appear to be determined by changes in the rate of distal volume delivery *per se*.

In similar microperfusion studies in the rat performed by Morgan [8], measurements of SNGFR were not influenced by increasing rates of distal lumenal perfusion with a solution containing sodium, chloride, potassium and phosphate. While these observations have been cited in refutation of the concept of distal tubular feedback control of SNGFR, it is equally possible that the perfusate in that study was lacking an important constituent of normal tubule fluid (or one that was present in perfusates utilized by other investigators when a relationship between distal perfusion and SNGFR was observed [5, 7]). In view of the fact that the calcium ion is known to exert an important influence on smooth muscle contractility and vascular reactivity [22–24], and recognizing that the distal perfusion solution used by Morgan was lacking in calcium whereas this ion was present in the solution utilized by Schnermann et al [5, 7] and one of the initial solutions used by us (ATF), it seemed reasonable to consider the possibility that alterations in the distal delivery of calcium might well play a significant role in activation of the feedback system. For this reason, a modified Ringer's solution with and without calcium was prepared and utilized in the present studies. Measurements of SNGFR during the maintenance of distal lumenal perfusion with calcium-free Ringer's solution were no different than those observed in nonperfused proximal tubules, and similar to those observed during distal perfusion with saline solution alone. Similarly, in studies by Schnermann et al [5], it is interesting to note that increased distal perfusion with mannitol and sodium sulfate solutions was not accompanied by alterations of SNGFR. In contrast, in the

present studies, the maintenance of distal perfusion with calcium-containing Ringer's solution and ATF was accompanied by measurements of SNGFR that were much lower than those observed in nonperfused tubules selected at random; i.e., activation of a feedback-mediated elevation of SNGFR seemed to have been minimized or prevented. Taken together, the present results suggest that some factor(s) associated with the reduced delivery of calcium may serve to activate the feedback mechanism and effect a regulatory elevation of SNGFR, and that the presence of calcium in distal nephron perfusate may well play a role in modulating the feedback control of SNGFR. However, it must be emphasized that the present results provide no insight into the possible influence of increased calcium delivery on SNGFR. In addition, since a modest increase in SNGFR was also observed during re-collection measurements from the same tubule following the cessation of perfusion with sodium chloride or calcium-free Ringer's solutions, it must be acknowledged that some function of distal volume or sodium delivery may also participate, at least in part, in any final determination of the level of activity of the feedback mechanism.

An alternative explanation for the observed increase of SNGFR in the same tubules following the cessation of distal perfusion may perhaps involve the finite magnitude of the period of time between initiation of the signal at the macula densa and the achievement of maximal afferent arteriolar vasodilation. During perfusion with ATF and calcium-containing solutions, it is possible that such a signal was not initiated until the perfusion pipet was withdrawn from the nephron; in contrast, during perfusion with isotonic saline and calcium-free Ringer's solution, the signal may well have been initiated earlier at the time when the macula densa was first exposed to the calcium-free solutions, i.e., at the start of distal perfusion. For this reason, the demonstration of a higher re-collection SNGFR in the latter circumstances may reflect nothing more than a dissimilarity in the total time of exposure of the distal nephron to one (or more) missing constituents of the perfusate. It is possible that the afferent vessels may not achieve minimum vascular tone for a minute or more following the introduction of intralumenal oil blockade of the proximal nephron.

The major significance of the present observations lies in the fact that they suggest that some function of altered distal calcium delivery may well play a possible mediatory role in regulation of a distal tubular-vascular feedback phenomenon. It is intriguing to speculate that this possibility is consistent with the known influence of calcium ion on smooth muscle contractility [22–24]; it is conceivable that some function of distal calcium delivery could serve as the major mediator and that additional factors are not required. In this regard, it is of interest to note that the contractile capability of isolated vessels is lost when they are bathed in calcium-free solutions and that such vessels become unresponsive to certain vasoconstrictor stimuli [24]. Taken together, the present results suggest, at least as a feasible alternative, that some function of distal calcium delivery may serve to influence the vascular reactivity of the afferent arteriole in a similarly direct fashion, and thereby participate in the operation of the distal tubule-afferent arteriole feedback mechanism. Of course, other possible explanations of the mechanism whereby afferent arteriolar tone may be influenced by changes of distal calcium delivery (or reabsorption) must be considered as well, and further studies will be required to determine whether the influence of calcium on vascular reactivity is direct or indirect, or primary or permissive in nature.

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